Review

Evolving Biomarkers in Kidney Transplantation

Maurizio Salvadori *, alberto rosati , Giuseppina Rosso

Posted Date: 17 April 2024

doi: 10.20944/preprints202404.1113.v1

Keywords: Kidney rejection; biomarkers; subclinical rejection; donor derived cell free DNA; gene expression profile; urinary cytokines

 doi:10.20944/preprints202404.1113.v1

Review

Evolving Biomarkers in Kidney Transplantation

Running title: Biomarkers in Transplantation

Maurizio Salvadori [1,*], Alberto Rosati [2] and Giuseppina Rosso [2]

[1] Department of Renal Transplantation Careggi University Hospital viale Pieraccini 18, 50139 Florence, Italy
[2] Division of Nephrology, San Giovanni di Dio Hospital, Florence, 50143, Toscana, Italy
alberto.rosati@uslcentro.toscana.it (A.R.); giuseppina.rosso@uslcentro.toscana.it (G.R.)
* Correspondence: maurizio.salvadori1@gmail.com; Tel./Fax: +0039-055-597151

Abstract: Precision medicine is essentially based on reliable and non-invasive biomarkers. Aim of this review has been to describe the newest biomarkers in the field of kidney transplantation and the kidney rejection, one of the commonest and severe complications. Standard of care tools to identify acute rejection are largely full of errors and of drawbacks. In recent years, new and reliable biomarkers have been found. They are avoid of risks, non-invasive and able to detect rejection even in those frequent cases in which acute rejection are clinically asymptomatic and not otherwise identifiable. In recent years, several biomarkers have been identified. Very recently have been found new relevant biomarkers able to diagnose rejection with high positive predictive value and low negative predictive value. These are the donor-derived cell free DNA found in the recipient, the gene expression profile of the donor found in the recipient and the urinary cytokines that express modification in the graft tissue. The aim of this study has been to find the most recent findings in the literature on this topic and to describe the utility and possible limitation of such new biomarkers on kidney rejection.

Keywords: Kidney rejection; biomarkers; subclinical rejection; donor derived cell free DNA; gene expression profile; urinary cytokines

Introduction and Definitions

Transplant medicine is slowly translating from the "evidence based medicine" to the "precision medicine". This fact is due to several factors and novel technologies among which biomarkers have a relevant role [1]. Since a long time several papers have been describing the role of biomarkers in renal transplantation [2].

Immunosuppression is a cornerstone in transplantation, but this therapy has to balance from insufficient drug therapy leading to acute or chronic rejection and excessive drug therapy leading to infections or malignancies. We have been delivering our immunosuppressive treatment with indiscriminate monitoring tools for decades in the past waiting for either clinically evident rejection to occur or clinically evident infection or malignancy to develop. The standard-of-care management to monitoring clinical events is shown in Table 1. These have several deficiencies or drawbacks.

Table 1. Standard-of-care management to monitoring clinical events and their drawbacks.

Serum creatinine testing	Creatinine is a lagging and non-specific marker of injury
Urine testing	Non-specific
Transplant ultrasonography	Non-specific
Screening and monitoring donor specific antibodies (DSA)	Not all DSAs are overtly pathogenic, many unknown non-HLA Abs
Drug level monitoring	Non-specific
Renal biopsy	Expensive and not without complications

 doi:10.20944/preprints202404.1113.v1

	Subject to sampling error and interpreter variability Histologic assessment has limitations

Serum creatinine testing is a lagging and not specific marker of injury as well as urine standard testing.

Screening and monitoring donor specific antibodies (DSAs) does not consider that not all DSAs are overtly pathogenic and that there are many unknown non-HLA antibodies.

Drug level monitoring is helpful but non-specific in the absence of other relevant signs.

Renal biopsy has always been considered the gold standard, but is expensive and not without risks/complications. In addition, it is subject to sampling errors and interpreter variability. Finally, the histologic assessment has several limitations, principally if conducted in not expert centers.

As a consequence, there is the need of non-invasive biomarkers that may be distinguished in diagnostic biomarkers, prognostic biomarkers, monitoring biomarkers and pharmacodynamics/response biomarkers, as shown in Table 2 [3–11].

Table 2. Overview of biomarker subtypes and assessment of kidney allografts.

Biomarker Type	**Biomarker Definition**	**Established Examples in Transplantation**	**Potential new Examples in Transplantation**
Diagnostic biomarker	A biomarker used to identify individuals with the disease or condition of interest or define a subset of the disease	Serum creatinine Proteinuria Hematuria DSA Signs of hemolysis Renal ultrasound examination Protocol or for cause biopsy histology	Urinary three gene mRNA expression signature and wide range of other suggested molecules [3,4] Wide range of urinary target proteins, like CXCL10 and CXCL9 [3] Blood 17-gene mRNA expression “kSORT” [5] Blood 200-gene mRNA expression “TruGraf” [6] Several blood and urine mRNAs [3] Molecular microscope for allograft pathology
Prognostic biomarker	A biomarker used to identify likelihood of a clinical event, disease recurrence, or progression	Serum creatinine Proteinuria DSA Protocol or for cause biopsy histology	Complement-fixing characteristics of DSA [7,8] Edmonton classifier for graft loss [9] Edmonton ABMR molecular score [10] GOCAR 13-gene set [11]
Monitoring biomarker	A biomarker measured serially and used to detect a change in the degree or extent of disease; monitoring biomarkers may also be used to indicate toxicity, assess safety, or provide evidence of exposure,	Serum creatinine Proteinuria Hematuria Immunosuppressive drug levels BKV/PCR Signs of hemolysis	There are currently no new monitoring biomarkers proposed in kidney transplantation

 doi:10.20944/preprints202404.1113.v1

	including exposures to medical products		
Pharmacodynamics/response biomarker	A biomarker used to show that a biologic response has occurred in an individual who has received an intervention or exposure	CD19/CD20 count with rituximab treatment DSA mean fluorescence index after AMMR treatment Post-treatment control biopsy histology	There are currently no new pharmacodynamics/response biomarkers proposed in kidney transplantation

In the story of a transplant, before than any disease activity is present risk/susceptibility biomarkers will facilitate the identification of high-risk patients who require closer follow up examinations, which are typically, performed using noninvasive diagnostic biomarkers. After a disease process is diagnosed, a prognostic biomarker estimates the erect of the disease and the chance of spontaneous resolution. A prognostic biomarker should be able to identify those patients who need treatment and those patients who will have a spontaneous disease resolution. If a patient with disease would have a poor outcome, the research for the best appropriate therapy will be based not only based on diagnosis and prognosis, but also based on predictive biomarkers and of safety/pharmacodynamics/response biomarkers and monitoring biomarkers, ideally noninvasive [1].

In this study, several evolving biomarkers in kidney transplantation will be treated as Donor-derived cell-free DNA (dd-cf DNA), Blood gene expression profiles (Trugraf study), Urine biomarkers (CXCL9, gene expression profiles).

Donor-Derived Cell-Free DNA (dd-cfDNA)

This biomarker is based on the fact that allograft cell injury leads to increase of dd-cfDNA in the bloodstream of the recipient [12]. It is a reliable marker of endothelial cell injury and can be elevated in rejection, infection and drug induced kidney injury [13–16]. It should be considered that there is a possible release of recipient-derived cfDNA by recipient's immunologic effector cells activated during rejection [15].

In addition, urinary cfDNA (so-called Transrenal DNA) is important. These molecules cross the kidney barrier and appear in the urine [17]. They reflect an increased burden of tissue injury and apoptosis [16]. May be both donor derived and recipient derived.

Overall, there are three clinically available assays:

Allosure (Care Dex)

Prospera (Natera)

TRAC (Virecor Eurofils)

Several questions are still open in understanding the significance of dd-cfDNA in kidney transplant recipients.

There is ongoing debate on whether relative or absolute quantification of dd-cfDNA is more reliable to detect acute transplant injury.

In a recent study from Osmadodja et al. [18], 22 kidney transplant patients underwent dd-cfDNA measurement either as percentage or as absolute. The study concludes that relative dd-cfDNA alone can lead to false negative and false positive results. The use of both absolute and relative dd-cfDNA is better to assure a higher reliability and interindividual comparability.

In a different study, Graver et al. [19] states that the potential benefits and pitfalls of dd-cfDNA are as shown in Table 3. In the same study the authors highlighted that dd-cfDNA are released by allograft into the bloodstream, the level is dependent on allograft health and that level < 0.5% are present in kidney transplant recipients without allograft injury. On the other hand, modification of

 doi:10.20944/preprints202404.1113.v1

dd-cfDNA is probably due to injury or rejection. Biopsy is still required to confirm pathological diagnosis.

Table 3. Benefits and pitfalls of the use of dd-cfDNA.

Potential Benefits	Pitfalls
Noninvasive blood biomarker	Fractional quantification affected by changes in rdcfDNA
Applicable to all solid organ transplantation	Does not exclude TCMR (if ddcfDNA normal)
Elevations may occur up to 30 days before histologic changes	Elevated in nonrejection pathologies associated with tissue injury (BKV, CVNI toxicity)
Absolute quantification of ddcfDNA not affected by changes in rdcfDNA	Not recommemded for use in early posttransplant period
Avoidance of protocol biopsy	No recommended flor use for 24 h post-biopsy
Avoidance of unnecessary biopsies	Confounded in pregnancy
Noninvasive diagnosis of AMR	Confounders in some repeat and multiorgan transplants
Assessment of response to rejection treatment	
Indicator for treatment of chronic active AMR	

In a study from Sigdel et al. [20] from the UCSF, the dd-cfDNA was assessed via massively multiplex PCR in 193 kidney transplant patients. All patients were biopsy matched: 38 had active rejection, 72 borderline rejection, 82 had a stable allograft and 25 with different types of injuries. The dd-cfDNA analyzed by the single nucleotide polymorphism (SNP) differentiated patients with active rejection from patients affected by all other conditions ($p<0.0001$) with high sensitivity (88.7%) and high specificity (77.6%) (Table 4). In this study, dd-cfDNA was not able to differentiate antibody mediated rejection (ABMR) from T cell mediated rejection (TCMR) ($p=0.855$). This is in contrast with the study of Bloom et al. [21]. In this study dd-cfDNA, levels are higher in ABMR (both chronic and acute) respect to TCMR (2.9% versus 1.2%). This study from the Circulating Donor Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients (DART) highlights that plasma levels of dd-cfDNA discriminate the active rejection pathogenesis.

Table 4. dd_cfDNA and diagnosis of rejection.

Acute rejection median	2,32%
Non-acute rejection median	0.47%

- AUC: 0.87
- Sensitivity: 88.7%
- Specificity: 72.6%
- PPV: 52,0%
- NPV: 95.1%

Two recent meta-analyses using the existing data, documented the relevance of dd-cfDNA in the diagnosis of kidney rejection [22,23].

The first one analyzed seven studies [24–28] and used the "Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines [29].

The median dd-cfDNA level was significantly higher in patients with ABMR respect to stable patients, while patients with TCMR did not have a different median dd-cfDNA compared to stable patients. Similar results were reported in the other meta-analysis [23], who analyzed nine studies.

In conclusion, dd-cfDNA can be a helpful marker for the diagnosis of ABMR in patients with suspected renal dysfunction, but not probably for patients with TCMR. An explanation of this fact is that ABMR results in a microvascular injury [30], with a release of free DNA after endothelial

 doi:10.20944/preprints202404.1113.v1

damage, while TCMR is essentially an interstitial injury that only rarely is associated with endovasculitis [31].

dd-cfDNA may also act as a prognostic tool. Even if several already cited studies documented that high levels of dd-cfDNA are diagnostic for ABMR rejections and not always distinguish TCMR rejection from stable kidneys, a recent study from Stites et al. [32]. This important study documents that high levels of dd-cfDNA identifies patients with TCMR1A and borderline allograft rejection at elevated risk of graft injury. The impact of any acute rejection on the risk of late allograft failure has been also documented by other studies [33,34]. Risk for a poor outcomes of kidney recipients with early posttransplant donor specific anti-HLA antibodies and high dd-cfDNA levels was documented also by the study of Cooper et al. [35].

In the study of Stites [32] were evaluated 79 patients with TCMR1A or borderline rejection. Rejections were evaluated with kidney biopsies, for cause, or for surveillance. Patients were stratified by dd-cfDNA >0.5% or dd-cfDNA < 0.5%. The % change in estimated glomerular filtration rate (eGFR) was measured 3-6 months after evaluation of dd-cfDNA levels and kidney biopsies. The decrease of eGFR, the presence of DSAs and the recurrence of rejection were statistically significant in patients with dd-cfDNA <0.5% respect to patients with dd-cfDNA >0.5%. The results are shown in Table 5.

Table 5. Patients with dd-cfDNA >0.5 were at increased risk of recurrent rejection, DSA detection, and eGFR decline over the following 3-6 months.

	Statistics	**Low (dd-cfDNA <0.5%**	**Hgh (dd-cfDNA > 0.5%**	**p-value**
dd-cfDNA value (%)	Mean (SD)	0.25 (0.087)	1.76 (1.40)	-
	Median	**0.21** (0.19, 0,29)	**1.40** (0.87, 2.02)	-
	Min, Max	0.19, 0.49	0.52, 6.70	-
% Change in eGFR	Mean (SD)	**-0.40** (18.149)	**-8.64** (11.98)	**0.0040**
	Median	0.00 (-0.92, 4,76	-7.50 (-16.22, -1.39)	
	Min, Max	-70.73, 33,.33	-37-50, 32.65	
Presence of DSAs		1/37 (2.7%)	17/42 (40.5%)	**<0.0001**
Recurrent Rejection		0/37 (0.0%)	9/42 (21.4%)	**0.0028**

dd-cfDNA is also important in determining the clinical outcomes of a transplant as determined by monitoring the kidney allografts with a longitudinal surveillance study. The study is called ADMIRAL and s reported by a paper from Bu et al. [36].

The study reports the data of 1094 patients from seven transplant centers. All patients received a single adult kidney, most of them from a deceased donor. The control of dd-cfDNA lasted 3 years posttransplant. In particular was performed an analysis of de novo DSA, of eGFR trajectories and of allograft rejections. Two previous studies [37,38] have already reported that a decline in eGFR is superior to other surrogate measures of long-term kidney transplant outcomes. The ADMIRAL study confirmed that persistently elevated dd-cfDNA (above 0.5%) predicted a >25% decline in eGFR over 3 years. Similarly, dd-cfDNA values >0.5% were associated with a nearly 3-fold increase in the risk of development of de novo donor specific antibody (DSA). Finally, significant elevations in dd-cfDNA were observed during rejection ahead of changes in serum creatinine. In conclusion, the ADMIRAL study demonstrates a broad utility of dd-cfDNA as a leading indicator ahead of clinical presentations of allograft injury, formation of dnDSA, eGFR decline and subclinical rejection.

 doi:10.20944/preprints202404.1113.v1

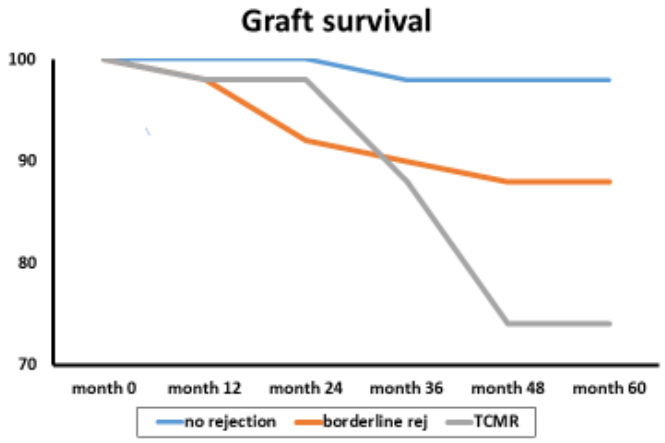
Treating of dd-cfDNA we have already highlighted the importance of diagnosing subclinical rejection by biomarkers in the absence of clinical signs.

Several studies document the importance of early recognizing the occurrence of early inflammation and sub-clinical rejection.

Nankivell et al. [39] evaluated in 551 renal transplant recipients, the clinical and pathological significance of borderline T cell mediated rejection against normal controls and acute TCMR.

The group of patients was followed for 60 months and borderline rejection was associated to renal dysfunction, acute tubular necrosis and chronic tubular atrophy. Additionally, patients with borderline rejection were associated to a reduced graft survival (Figure 1).

Figure 1 Graft survival according the rejection type



Similarly, subclinical inflammation phenotypes and worse long-term renal outcomes have been observed in some group of patients as pediatric kidney transplantation [40] and kidney transplant recipients with a rapid steroid withdrawal protocol [41]. Hence the importance of new biomarkers in addition to dd-cfDNA.

Several studies recognize the importance of gene profile in the diagnosing of early inflammation and subclinical rejection after kidney transplantation.

Gene Expression Profile as Biomarkers

Fridewald et al. [42] documented the development and clinical validity of a novel blood-based molecular biomarker for the diagnosis of subclinical acute rejection following kidney transplantation.

Several previous studies had documented that the gene expression profiles (GEPs) in both urine and blood could detect kidney transplant rejection. The CTOT-04 study [4] documented that a 3-gene signature in urine samples was able to detect kidney rejection. In a similar way, the CTOT-01 study [43] documented that urine CXCL9 protein revealed the existing of rejection. In the CTOT-08 study [42], Fridewald et al. demonstrated the clinical validity of the clinical significance of both the clinical phenotype (CP) and GEP of subclinical acute rejection within the first 12 months on the composite clinical endpoint. The same study documented in these subjects the development of de novo DNAs by the 24 months. In particular, in the CTOT-08 study, the gene expression profile consisted of 120 genes that are up and down regulated and specifically chosen to distinguish stable normal biopsy from subclinical rejection.

In a different study, Zhang et al. [44], examined 191 kidney transplant patients from the prospective Genomic of Chronic Allograft Rejection (GoCAR) study [45], who underwent surveillance biopsies over 2 years and identified patients with subclinical or borderline acute cellular rejection (ACR) at three months (ACR-3) post transplant. These patients had a subsequent worse outcome with decline in renal function and decreased graft survival. Using a RNA sequencing

 doi:10.20944/preprints202404.1113.v1

analysis, the authors identified a 17-gene signature (Table 6). This TREx assay based on the 17-gene set achieved PPV of 0.79 and NPV of 0.98 for subclinical ACR diagnosis.This set was validated and represents a peripheral blood gene expression signature to diagnose subclinical acute rejection and to risk stratify kidney transplant recipients.

Table 6. Seventeen –gene set for 3-month ACR diagnosis.

Symbol	RefSeq	Name	P-value
ZMAT1	NM_001011657	Zinc finger, matrin type 1	0.01
ETAA1	NM_019002	Ewing tumor.associated antigen 1	0.04
ZNF493	NM_001076678	Zinc finger protein 493	0.002
CCDC82	NM_024725	Coiled-coil domain containing 82	0.02
NFYB	NM_006166	Nuclear transcription factor Y, β	0.03
SENP7	NM_001077203	SUMO1/sentrin specific peptidase 7	<0.001
CLK1	NM_001162407	CDC-like kinase 1	0.01
SENP6	NM_001100409	SUMO1/sentrin specific peptidase 6	0.01
C1GALT1C1	NM_001011551	C1GALT1-specific chaperone 1	0.01
SPCS3	NM_021928	Signal peptidase complex subunit 3 homolog (S,. cerevisiae)	0.03
MAP1A	NM_002373	Microtubule-associated protein 1A	0.01
EFTUD2	NM_001142605	Elongation factor Tu GTP binding domain containing 2	0.001
AP1M1	NM_001130524	Adaptor-related protein complex 1, mu 1 subunit	<0001
ANXA5	NM_001154	Annexin A5	<0.001
TSC22D1	NM_001243797	TSC22 domain family, member 1	0.01
F13A1	NM_000129	Coagulation factor XIII, A1 polypeptide	0.02
TUBB1	NM_030773	Tubulin, β1 class VI	0.03

An important blood gene expression classifier in the field of kidney transplantation is the TruGraf. It represents a novel molecular biomarker for managing kidney transplant recipients with stable renal function [46]. The TruGraf algorithm is a DNA microarray-based gene expression algorithm that analyzes the gene expression profile of 120 genes. In the original study it was designed to avoid surveillance biopsies and was validated in multiple cohorts. Simultaneous blood tests and clinical assessments have been performed on 192 patients from seven transplant centers [46]. Evaluating the results of TruGraf blood test and compared with clinical phenotype in 99 kidney transplant recipients with stable renal function and biopsy confirmed phenotypes and in 63 kidney transplant patients with stable renal function but with per protocol biopsy documenting subclinical rejection, the accuracy was 74% in patients without rejection and 80% in patients with rejection. The overall negative predictive value was 89% and the positive predictive value 48% with sensitivity of 71% and specificity of 75%. The conclusion of the study was in favor of a clinical decision without unnecessary surveillance biopsies basing on an accurate gene expression profile (GEP).

Basing on TruGraf method Heilman et al. [47] conducted a prospective study obtaining peripheral blood at five time points in the first year post transplant to obtain GEP. Overall, 240 kidney transplant patients have been enrolled and stratified into three groups according the absence or presence of one or more GEP. Presence of multiple GEP correlated with a poorer histological aspects, lower eGFR and higher death censored graft loss.

We have described the relevance in diagnosing subclinical rejection of dd-cfDNA and the gene expression profile. A question is whether blood gene expression assays and donor-derived cell free DNA may be used together to diagnose subclinical rejection. Park et al. [48] have provided an answer to this question recently.

 doi:10.20944/preprints202404.1113.v1

In this study, the authors enrolled 208 subjects for a total of 428 biopsies samples. The study was a post hoc analysis of the clinical Trial in Organ Transplantation 08. Surveillance biopsies have been performed from month 2 to month 6 post-transplant and at month 12 and 24.

Patients were simultaneously followed by gene expression profile assay (TruGraf method); donor derived cfDNA and combined tests. Observing the diagnosis of subclinical rejection, the authors reported a PPV of 47% with gene profile, of 56% with dd-cfDNA, of 81% with the combined tests.

A NPV was 82% with gene profile, 84% with dd-cfDNA and 88% with combined tests. The area under the receiver operating characteristic curve (**AUROC**) values was similar with each method. Overall, the GEP was better at detecting cellular rejection and dd-cfDNA was better in detecting antibody rejection.

In conclusion, donor derived cell free DNA and gene expression profiles provide a less invasive monitoring strategy for subclinical rejection with different detection of antibody and cell mediated rejection.

Urinary RNA Profile for the Diagnosis of Rejection

Independently from the information on clinical or subclinical rejection that can be drawn from the blood, the urine too may allow the rejection diagnosis of a kidney graft in a non-invasive method.

Li et al. [49] comparing the urine specimen from 22 kidney graft with a biopsy confirmed acute rejection with 63 grafts without biopsy-confirmed acute rejection found higher levels of perforin mRNA and granzyme B mRNA in patients with biopsy confirmed acute rejection. Both mRNA encode the cytotoxic proteins.

The authors divided the patients in four groups (acute rejection, stable graft function, other pathological findings not rejection related, and chronic rejection). The levels of perforin mRNA were significantly higher in patients with acute rejection than patients with stable renal function ($p<0.001$), patients with other findings ($p<0.001$) and patients with chronic rejection ($p=0.03$).

This was not the case of granzyme B mRNA levels, which were not able to distinguish patients with acute rejection from patients with chronic rejection ($p =0.12$).

The authors conclude that urinary mRNA levels of perforin and granzyme B are a useful noninvasive tool for the diagnosis of acute rejection, but levels of granzyme B mRNA were not able to distinguish acute rejection from chronic rejection.

We have already cited the study of Suthanthiran et al. [4]. One year later the same authors published another more extensive study on the urinary mRNA profile and acute rejection in kidney transplant recipients [50]. Overall, a total of 4300 urine samples were collected from 485 patients for urinary-cell messenger RNA (mRNA) repeatedly after transplantation and at the time of kidney allograft biopsy. Urinary mRNA has been examined for CD3 ε perforin, granzyme B, interferon-inducible protein 10 (IP-10), CXCR3, CD103, transforming growth factor β (TGF β) and proteinase inhibitor 9. Patients were divided into three groups (acute cellular rejection, No rejection; and stable function in patients which did not receive renal biopsy). The mRNA levels of CD ε perforin, granzyme B, and IP-10 differed significantly among the three groups ($p< =.001$), but not the levels of CXCR3 ($p = 0.06$), CD 103 ($p = 0.13$), TGFβ ($p = 0.11$), and proteinase inhibitor 9 ($p = 0.38$).

The authors conclude that CD3 εmRNA, IP-10 mRNA and 18S rRNA levels in urinary cells appear to be diagnostic of acute rejection in kidney allografts.

In another study [51], messenger RNA (mRNA) for *FOXP3*, a specification and functional factor for regulatory T lymphocytes, and mRNA for CD25, CD3εperforin, and 18S ribosomal RNA (rRNA) were examined in urine specimen of patients with acute rejection, with chronic allograft nephropathy and in patients with a normal renal biopsy. In particular, was examined the relationship of these mRNA levels and acute rejection, rejection reversal and graft failure. mRNA levels for all the factors studied were significantly higher in acute rejection than chronic rejection and stable graft function ($p<0.001$). *FOXP3* mRNA was the only one factor that related with reversibility. Patients with higher *FOXP3* mRNA had lower possibility to have a reversible rejection. Similarly, patients with higher

 doi:10.20944/preprints202404.1113.v1

FOXP3 mRNA had higher risk of graft loss. On the contrary, mRNA for CD25, CD3εperforin, and 18S ribosomal RNA (rRNA) did not had relationship with rejection reversibility and risk of graft loss.

In the already cited study from Hricik et al. [43], have been enrolled 280 kidney transplant patients, principally from living donors to evaluate and compare urinary biomarkers useful for a sure diagnosis of acute rejection. At the time of collecting urine, all patients received a for-cause renal biopsy. Urinary samples were examined for sediment RNA already known to be elevated for acute rejection as CCR1, CCR5, CXCR3, CCL5, CXCL9 (cytokine induced by interferon gamma), CXCL10, IL-8, perforin and granzyme B [52–54].

Using a correlation analysis, many of these substances highly correlated and showed interdependence. CXCL9 and CXCL10 documented the higher significant in diagnosing acute rejection. CXCL 10 was higher in patients with acute rejection and in patients with infections. Therefore, in the author's opinion CXCL9 is the protein with the higher significance in diagnosing acute rejection and in distinguishing acute rejection from other renal injuries. In detail, CXCL9 in acute rejection diagnosis had an area under the curve (AUC) of 0.856, a PPV of 67.6% and a NPV of 92%. The addition of CXCL10 did not modified the results.

However, the utility on the use of urinary cytokines as noninvasive biomarkers is the diagnosis of acute rejection is discussed by other studies, which found several controversies. In a French retrospective study [55], reviewing 329 transplanted patients, the utility of urinary CXCL9 and CXCL10 is discussed as a noninvasive method to diagnosing allograft rejection. To be considered in the retrospective study, all patients should have an allograft biopsy specimen, concomitant urine samples and blood research for BKV infection. Indeed, the study documented that a similar elevation of these urinary cytokines is found in the case of acute rejection as well as in the case of BKV infection and in the case of urinary tract infection frequently observed in kidney transplant subjects.

In a different and very, recent study [56] was evaluated the utility of urinary CXCL 10 to monitoring the renal allograft. In the study, patients were divided in two arms. The intervention arm (120 patients) and the control arm (121 patients. In both arms urine for detecting CXCL 10 were collected at month 1, 3 and 6. If elevated, renal biopsy was made and the subsequent treatment of rejection in intervention arm. In the control arm, the results were concealed. At 1 year were evaluated the death censored graft loss, the existence of an acute rejection, the presence of de novo DSA and the presence of an eGFR < 25 ml/min. Considering the combined endpoint at 1 year, the intervention arm and the control arm did not differ (51% vs 49%) and the study could not demonstrate the beneficial effect of urine CXCL 10 monitoring.

On the other hand, another study from Hricik et al. for the Clinical Trials in Organ Transplantation-09 Consortium [57] documented the usefulness of cytokines and other tools in predicting high-risk patients. The study had the aim of analyzing the safety of Tacrolimus withdrawal in immune-quiescent kidney transplant recipients. Overall, were randomized 21 patients, 14 in the tacrolimus withdrawal group, 7 in the control group. The study was terminated prematurely because of the high risk of acute rejection in the tacrolimus withdrawal group. High mismatches pretransplant, donor-reactive IFN-γ ELISPOT assay and high levels of urinary CXCL9 were all predictive of the development of acute rejection or/and development of DSAs in the tacrolimus withdrawal arm.

Table 7 highlights the main challenges in the field of finding new biomarkers.

Table 7. Main challenges in finding new biomarkers.

• The rapidly evolving field of biomarker-informed precision medicine will be led astray if clinicians do not continually inform the data to turn it into useful information that can help patients
• This principle is not new, but the allure of a new and "easy" test result that gives us "all the answers" is seductive
• We must remember our value to the patient remains being the link between the stream of data and the clinical reality

 doi:10.20944/preprints202404.1113.v1

- Lastly…..we are dealing with complex patients with complicated intersections of disease states that are in near constant flux

Conclusions

In conclusion, multiple new non-invasive and invasive biomarkers are changing the paradigm of rejection diagnosis and immunosuppression management.

Further refinement of the proper context of use and interpretation of these tests will shape patient care as transplantation moves further into the field of personalized medicine.

Well designed, interventional clinical trials using these tools are the next logical step in development.

Authors contribution: Salvadori M, Rosati A and Rosso G contributed equally to the manuscript; Salvadori M designed the study. Rosso G collected the data from the literature; Salvadori M and Rosatin A analyzed the collected data; Salvadori M, Rosati A and Rosso G wrote the manuscript. All the authors performed and approved the last revision.

Conflict of interest statement: Maurizio Salvadori, Alberto Rosati and Giuseppina Rosso do not have any conflict of interest in relation with the manuscript.

References

1. Naessens, Anglicheau D. Precision Transplant Medicine: Biomarkers to the Rescue. *J Am Soc Nephrol*. **2018**; *29*:24-34
2. Salvadori M, Tsalouchos A. Microbiota, renal disease and renal transplantation. *World J Transplant.* **2021**;*11*:16-36
3. Anglicheau D, Naesens M, Essig M, Gwinner W, Marquet P. Establishing Biomarkers in Transplant Medicine: A Critical Review of Current Approaches. *Transplantation.* **2016**;*100*:2024-2038
4. Suthanthiran M, Schwartz JE, Ding R, Abecassis M, Dadhania D, Samstein B, Knechtle SJ, Friedewald J, Becker YT, Sharma VK, Williams NM, Chang CS, Hoang C, Muthukumar T, August P, Keslar KS, Fairchild RL, Hricik DE, Heeger PS, Han L, Liu J, Riggs M, Ikle DN, Bridges ND, Shaked A; Clinical Trials in Organ Transplantation 04 (CTOT-04) Study Investigators. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. *Transplantation.* **2012**;*93*:1136-1146
5. Roedder S, Sigdel T, Salomonis N, Hsieh S, Dai H, Bestard O, Metes D, Zeevi A, Gritsch A, Cheeseman J, Macedo C, Peddy R, Medeiros M, Vincenti F, Asher N, Salvatierra O, Shapiro R, Kirk A, Reed EF, Sarwal MM. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study. *PLoS Med.* **2014**;**11**:e1001759
6. Kurian SM, Williams AN, Gelbart T, Campbell D, Mondala TS, Head SR, Horvath S, Gaber L, Thompson R, Whisenant T, Lin W, Langfelder P, Robison EH, Schaffer RL, Fisher JS, Friedewald J, Flechner SM, Chan LK, Wiseman AC, Shidban H, Mendez R, Heilman R, Abecassis MM, Marsh CL, Salomon DR. Molecular classifiers for acute kidney transplant rejection in peripheral blood by whole genome gene expression profiling. *Am J Transplant.* **2014**;*14*:1164-1172
7. Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen JP, Mooney N, Suberbielle C, Frémeaux-Bacchi V, Méjean A, Desgrandchamps F, Anglicheau D, Nochy D, Charron D, Empana JP, Delahousse M, Legendre C, Glotz D, Hill GS, Zeevi A, Jouven X. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med.* **2013**;*369*:1215-1226
8. Sicard A, Ducreux S, Rabeyrin M, Couzi L, McGregor B, Badet L, Scoazec JY, Bachelet T, Lepreux S, Visentin J, Merville P, Fremeaux-Bacchi V, Morelon E, Taupin JL, Dubois V, Thaunat O. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. *J Am Soc Nephrol.* **2015**; *26* :457-467.
9. Einecke G, Reeve J, Sis B, Mengel M, Hidalgo L, Famulski KS, Matas A, Kasiske B, Kaplan B, Halloran PF. A molecular classifier for predicting future graft loss in late kidney transplant biopsies. *J Clin Invest.* **2010**;*120*:1862-1872
10. Loupy A, Lefaucheur C, Vernerey D, Chang J, Hidalgo LG, Beuscart T, Verine J, Aubert O, Dubleumortier S, Duong van Huyen JP, Jouven X, Glotz D, Legendre C, Halloran PF. Molecular microscope strategy to improve risk stratification in early antibody-mediated kidney allograft rejection. *J Am Soc Nephrol.* **2014** ;*25*:2267-2277
11. O'Connell PJ, Zhang W, Menon MC, Yi Z, Schröppel B, Gallon L, Luan Y, Rosales IA, Ge Y, Losic B, Xi C, Woytovich C, Keung KL, Wei C, Greene I, Overbey J, Bagiella E, Najafian N, Samaniego M, Djamali A, Alexander SI, Nankivell BJ, Chapman JR, Smith RN, Colvin R, Murphy B. Biopsy transcriptome expression

profiling to identify kidney transplants at risk of chronic injury: a multicentre, prospective study. *Lancet.* **2016**;*388*: 983-993.
12. Gielis EM, Ledeganck KJ, De Winter BY, Del Favero J, Bosmans JL, Claas FH, Abramowicz D, Eikmans M. Cell-Free DNA: An Upcoming Biomarker in Transplantation. *Am J Transplant.* **2015**;**15**: 2541-2551
13. Beck J, Bierau S, Balzer S, Andag R, Kanzow P, Schmitz J, Gaedcke J, Moerer O, Slotta JE, Walson P, Kollmar O, Oellerich M, Schütz E. Digital droplet PCR for rapid quantification of donor DNA in the circulation of transplant recipients as a potential universal biomarker of graft injury. *Clin Chem.* **2013**; *59* :1732-1741
14. Zhang J, Tong KL, Li PK, Chan AY, Yeung CK, Pang CC, Wong TY, Lee KC, Lo YM. Presence of donor- and recipient-derived DNA in cell-free urine samples of renal transplantation recipients: urinary DNA chimerism. *Clin Chem.* **1999**; *45*: 1741-1746.
15. García Moreira V, Prieto García B, Baltar Martín JM, Ortega Suárez F, Alvarez FV. Cell-free DNA as a noninvasive acute rejection marker in renal transplantation. *Clin Chem.* **2009**; *55* :1958-1966
16. Sigdel TK, Vitalone MJ, Tran TQ, Dai H, Hsieh SC, Salvatierra O, Sarwal MM. A rapid noninvasive assay for the detection of renal transplant injury. *Transplantation*. **2013**; *96*: 97-101
17. Botezatu I, Serdyuk O, Potapova G, Shelepov V, Alechina R, Molyaka Y, Ananév V, Bazin I, Garin A, Narimanov M, Knysh V, Melkonyan H, Umansky S, Lichtenstein A. Genetic analysis of DNA excreted in urine: a new approach for detecting specific genomic DNA sequences from cells dying in an organism. *Clin Chem.* **2000**; *46*:1078-1084.
18. Osmanodja B, Akifova A, Budde K, Choi M, Oellerich M, Schütz E, Beck J. Absolute or Relative Quantification of Donor-derived Cell-free DNA in Kidney Transplant Recipients: Case Series. *Transplant Direct.* 2021; *7*: e778
19. Graver AS, Lee D, Power DA, Whitlam JB. Understanding Donor-derived Cell-free DNA in Kidney Transplantation: An Overview and Case-based Guide for Clinicians. *Transplantation.* **2023**;*107*:1675-1686
20. Sigdel TK, Archila FA, Constantin T, Prins SA, Liberto J, Damm I, Towfighi P, Navarro S, Kirkizlar E, Demko ZP, Ryan A, Sigurjonsson S, Sarwal RD, Hseish SC, Chan-On C, Zimmermann B, Billings PR, Moshkevich S, Sarwal MM. Optimizing Detection of Kidney Transplant Injury by Assessment of Donor-Derived Cell-Free DNA via Massively Multiplex PCR. *J Clin Med.* **2018**;*8*:19
21. Bloom RD, Bromberg JS, Poggio ED, Bunnapradist S, Langone AJ, Sood P, Matas AJ, Mehta S, Mannon RB, Sharfuddin A, Fischbach B, Narayanan M, Jordan SC, Cohen D, Weir MR, Hiller D, Prasad P, Woodward RN, Grskovic M, Sninsky JJ, Yee JP, Brennan DC; Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Active Rejection in Kidney Transplant Recipients (DART) Study Investigators. Cell-Free DNA and Active Rejection in Kidney Allografts. *J Am Soc Nephrol.* **2017**;*28*: 2221-2232
22. Wijtvliet VPWM, Plaeke P, Abrams S, Hens N, Gielis EM, Hellemans R, Massart A, Hesselink DA, De Winter BY, Abramowicz D, Ledeganck KJ. Donor-derived cell-free DNA as a biomarker for rejection after kidney transplantation: a systematic review and meta-analysis. *Transpl Int.* **2020**; *33*:1626-1642
23. Xiao H, Gao F, Pang Q, Xia Q, Zeng X, Peng J, Fan L, Liu J, Wang Z, Li H. Diagnostic Accuracy of Donor-derived Cell-free DNA in Renal-allograft Rejection: A Meta-analysis. *Transplantation.* **2021**;*105*:1303-1310
24. Oellerich M, Shipkova M, Asendorf T, Walson PD, Schauerte V, Mettenmeyer N, Kabakchiev M, Hasche G, Gröne HJ, Friede T, Wieland E, Schwenger V, Schütz E, Beck J. Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: Results from a prospective observational study. *Am J Transplant.* **2019**;*19*:3087-3099
25. Whitlam JB, Ling L, Skene A, Kanellis J, Ierino FL, Slater HR, Bruno DL, Power DA. Diagnostic application of kidney allograft-derived absolute cell-free DNA levels during transplant dysfunction. *Am J Transplant.* **2019**;*19*:1037-1049
26. Zhang H, Zheng C, Li X, Fu Q, Li J, Su Q, Zeng L, Liu Z, Wang J, Huang H, Xu B, Ye M, Liu L, Wang C. Diagnostic Performance of Donor-Derived Plasma Cell-Free DNA Fraction for Antibody-Mediated Rejection in Post Renal Transplant Recipients: A Prospective Observational Study. *Front Immunol.* **2020**;*11*: 342.
27. Bromberg JS, Brennan DC, Poggio E, Bunnapradist S, Langone A, Sood P, Matas AJ, Mannon RB, Mehta S, Sharfuddin A, Fischbach B, Narayanan M, Jordan SC, Cohen DJ, Zaky ZS, Hiller D, Woodward RN, Grskovic M, Sninsky JJ, Yee JP, Bloom RD. Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications. *J Appl Lab Med.* **2017**;*2*: 309-321
28. Huang E, Sethi S, Peng A, Najjar R, Mirocha J, Haas M, Vo A, Jordan SC. Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. *Am J Transplant.* **2019**;*19*:1663-1670
29. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* **2000**; *283*: 2008-2012
30. Lefaucheur C, Loupy A. Antibody-Mediated Rejection of Solid-Organ Allografts. *N Engl J Med.* **2018**;*379*: 2580-2582

 doi:10.20944/preprints202404.1113.v1

31. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, Nankivell BJ, Halloran PF, Colvin RB, Akalin E, Alachkar N, Bagnasco S, Bouatou Y, Becker JU, Cornell LD, Duong van Huyen JP, Gibson IW, Kraus ES, Mannon RB, Naesens M, Nickeleit V, Nickerson P, Segev DL, Singh HK, Stegall M, Randhawa P, Racusen L, Solez K, Mengel M. The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* **2018**;*18*: 293-307
32. Stites E, Kumar D, Olaitan O, John Swanson S, Leca N, Weir M, Bromberg J, Melancon J, Agha I, Fattah H, Alhamad T, Qazi Y, Wiseman A, Gupta G. High levels of dd-cfDNA identify patients with TCMR 1A and borderline allograft rejection at elevated risk of graft injury. *Am J Transplant.* **2020**;*20*: 2491-2498
33. Pallardó Mateu LM, Sancho Calabuig A, Capdevila Plaza L, Franco Esteve A. Acute rejection and late renal transplant failure: risk factors and prognosis *Nephrol Dial Transplant.* **2004**;*19* Suppl 3:iii38-42
34. Jevnikar AM, Mannon RB. Late kidney allograft loss: what we know about it, and what we can do about it. *Clin J Am Soc Nephrol.* **2008**;*3* Suppl 2: S56-S67
35. Cooper JE, Gralla K, Chan K Clinical significance of post kidney transplant de novo DSA in otherwise stable grafts *Clin Transpl* **2011**; *35*: 359-364.
36. Bu L, Gupta G, Pai A, Anand S, Stites E, Moinuddin I, Bowers V, Jain P, Axelrod DA, Weir MR, Wolf-Doty TK, Zeng J, Tian W, Qu K, Woodward R, Dholakia S, De Golovine A, Bromberg JS, Murad H, Alhamad T. Clinical outcomes from the Assessing Donor-derived cell-free DNA Monitoring Insights of kidney Allografts with Longitudinal surveillance (ADMIRAL) study. *Kidney Int.* **2022**;*101*:793-803
37. Clayton PA, Lim WH, Wong G, Chadban SJ. Relationship between eGFR Decline and Hard Outcomes after Kidney Transplants. *J Am Soc Nephrol*. **2016**; *27*: 3440-3446
38. Faddoul G, Nadkarni GN, Bridges ND, Goebel J, Hricik DE, Formica R, Menon MC, Morrison Y, Murphy B, Newell K, Nickerson P, Poggio ED, Rush D, Heeger PS; CTOT-17 consortium. Analysis of Biomarkers Within the Initial 2 Years Posttransplant and 5-Year Kidney Transplant Outcomes: Results From Clinical Trials in Organ Transplantation-17. *Transplantation.* **2018**;*102*: 673-680
39. Nankivell BJ, Agrawal N, Sharma A, Taverniti A, P'Ng CH, Shingde M, Wong G, Chapman JR. The clinical and pathological significance of borderline T cell-mediated rejection. *Am J Transplant.* **2019**;*19*:1452-1463
40. Seifert ME, Yanik MV, Feig DI, Hauptfeld-Dolejsek V, Mroczek-Musulman EC, Kelly DR, Rosenblum F, Mannon RB. Subclinical inflammation phenotypes and long-term outcomes after pediatric kidney transplantation. *Am J Transplant.* **2018** ;*18*: 2189-2199
41. Mehta R, Bhusal S, Randhawa P, Sood P, Cherukuri A, Wu C, Puttarajappa C, Hoffman W, Shah N, Mangiola M, Zeevi A, Tevar AD, Hariharan S. Short-term adverse effects of early subclinical allograft inflammation in kidney transplant recipients with a rapid steroid withdrawal protocol. *Am J Transplant.* **2018** ;*18* : 1710-1717
42. Friedewald JJ, Kurian SM, Heilman RL, Whisenant TC, Poggio ED, Marsh C, Baliga P, Odim J, Brown MM, Ikle DN, Armstrong BD, Charette JI, Brietigam SS, Sustento-Reodica N, Zhao L, Kandpal M, Salomon DR, Abecassis MM; Clinical Trials in Organ Transplantation 08 (CTOT-08). Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant. *Am J Transplant.* **2019**;*19*: 98-109.
43. Hricik DE, Nickerson P, Formica RN, Poggio ED, Rush D, Newell KA, Goebel J, Gibson IW, Fairchild RL, Riggs M, Spain K, Ikle D, Bridges ND, Heeger PS; CTOT-01 consortium.Multicenter validation of urinary CXCL9 as a risk-stratifying biomarker for kidney transplant injury. *Am J Transplant*. **2013**;*13*: 2634-2644
44. Zhang W, Yi Z, Keung KL, Shang H, Wei C, Cravedi P, Sun Z, Xi C, Woytovich C, Farouk S, Huang W, Banu K, Gallon L, Magee CN, Najafian N, Samaniego M, Djamali A, Alexander SI, Rosales IA, Smith RN, Xiang J, Lerut E, Kuypers D, Naesens M, O'Connell PJ, Colvin R, Menon MC, Murphy B.
A Peripheral Blood Gene Expression Signature to Diagnose Subclinical Acute Rejection *J Am Soc Nephrol.* **2019**; *30* :1481-1494
45. Yazdani S, Naesens M. Foretelling Graft Outcome by Molecular Evaluation of Renal Allograft Biopsies: The GoCAR Study. *Transplantation.* **2017**;*101*: 5-7
46. Marsh CL, Kurian SM, Rice JC, Whisenant TC, David J, Rose S, Schieve C, Lee D, Case J, Barrick B, Peddi VR, Mannon RB, Knight R, Maluf D, Mandelbrot D, Patel A, Friedewald JJ, Abecassis MM, First MR. Application of TruGraf v1: A Novel Molecular Biomarker for Managing Kidney Transplant Recipients With Stable Renal Function. *Transplant Proc.* **2019**; *51*:722-728
47. Heilman RL, Fleming JN, Mai M, Smith B, Park WD, Holman J, Stegall MD. Multiple abnormal peripheral blood gene expression assay results are correlated with subsequent graft loss after kidney transplantation. *Clin Transplant.* **2023** ; *37*: e14987
48. Park S, Guo K, Heilman RL, Poggio ED, Taber DJ, Marsh CL, Kurian SM, Kleiboeker S, Weems J, Holman J, Zhao L, Sinha R, Brietigam S, Rebello C, Abecassis MM, Friedewald JJ. Combining Blood Gene Expression and Cellfree DNA to Diagnose Subclinical Rejection in Kidney Transplant Recipients. *Clin J Am Soc Nephrol.* **2021**;*16*:1539-1551

 doi:10.20944/preprints202404.1113.v1

49. Li B, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med.* **2001**; *344*: 947-954
50. Suthanthiran M, Schwartz JE, Ding R, Abecassis M, Dadhania D, Samstein B, Knechtle SJ, Friedewald J, Becker YT, Sharma VK, Williams NM, Chang CS, Hoang C, Muthukumar T, August P, Keslar KS, Fairchild RL, Hricik DE, Heeger PS, Han L, Liu J, Riggs M, Ikle DN, Bridges ND, Shaked A; Clinical Trials in Organ Transplantation 04 (CTOT-04) Study Investigators. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. *N Engl J Med.* **2013** ; *369* :20-31
51. Muthukumar T, Dadhania D, Ding R, Snopkowski C, Naqvi R, Lee JB, Hartono C, Li B, Sharma VK, Seshan SV, Kapur S, Hancock WW, Schwartz JE, Suthanthiran M. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. *N Engl J Med.* **2005**; *35 3*:2342-2351
52. Ho J, Wiebe C, Gibson IW, Rush DN, Nickerson PW. Immune monitoring of kidney allografts. *Am J Kidney Dis.* **2012**; *60*: 629-640
53. Panzer U, Reinking RR, Steinmetz OM, Zahner G, Sudbeck U, Fehr S, Pfalzer B, Schneider A, Thaiss F, Mack M, Conrad S, Huland H, Helmchen U, Stahl RA. CXCR3 and CCR5 positive T-cell recruitment in acute human renal allograft rejection. *Transplantation.* **2004**; *78* :1341-1350
54. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B, Mackay CR. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest.* **1998** ; *101*: 746-754
55. Tinel C, Devresse A, Vermorel A, Sauvaget V, Marx D, Avettand-Fenoel V, Amrouche L, Timsit MO, Snanoudj R, Caillard S, Moulin B, Olagne J, Essig M, Gwinner W, Naesens M, Marquet P, Legendre C, Terzi F, Rabant M, Anglicheau D. Development and validation of an optimized integrative model using urinary chemokines for noninvasive diagnosis of acute allograft rejection. *Am J Transplant.* **2020**; 20: 3462-3476
56. Hirt-Minkowski P, Handschin J, Stampf S, Hopfer H, Menter T, Senn L, Hönger G, Wehmeier C, Amico P, Steiger J, Koller M, Dickenmann M, Schaub S. Randomized Trial to Assess the Clinical Utility of Renal Allograft Monitoring by Urine CXCL10 Chemokine. *J Am Soc Nephrol*. **2023**; *34* :1456-1469
57. Hricik DE, Formica RN, Nickerson P, Rush D, Fairchild RL, Poggio ED, Gibson IW, Wiebe C, Tinckam K, Bunnapradist S, Samaniego-Picota M, Brennan DC, Schröppel B, Gaber O, Armstrong B, Ikle D, Diop H, Bridges ND, Heeger PS; Clinical Trials in Organ Transplantation-09 Consortium. Adverse Outcomes of Tacrolimus Withdrawal in Immune-Quiescent Kidney Transplant Recipients. *J Am Soc Nephrol.* **2015**; *26*: 3114-3122.